loss of bone due to immobilization. Nevertheless, calcium supplements *will* prevent the loss of bone due to inadequate calcium intake. Our mistake has been to generalize from studies in the immediate postmenopause, which is dominated by the readjustments produced by estrogen withdrawal, to periods either earlier or later, for which there is ample evidence that a high calcium intake is bone sparing.[18-20]

Although it is never too late to start to assure an adequate calcium intake, it must also be acknowledged that the roots of the problem go deep. The building of an optimal bone mass, particularly during the teen years and young adulthood, is of critical importance. Many teenaged girls today have such low calcium intakes[21] that there is no possibility that they can repeal the law of the conservation of mass and make much of a skeleton from the raw materials they provide their bodies. It is today's teenagers with low calcium intakes who are likely to be our hip fracture patients in 60-plus years from now. We are doomed to a perpetual game of catch-up until we can implement effective populationwide strategies to increase the level of calcium intake. This is an achievable goal, just as years ago ways were found to increase intakes of such trace nutrients as fluoride and iodine. If one is tempted to think about this as medicating the population, it may be instructive to bear two facts in mind: the primitive human calcium intake—the one, presumably, to which our physiology is adapted—is well in excess of 1,500 mg per day[22]; and the natural food sources of our closest primate relatives provide them with diets containing roughly four times the calcium nutrient density of a typical first-world human diet.

Next, there is the importance of maintaining physical activity, both before and after a fracture. Our skeletons are, after all, mechanical systems, and the principal intrinsic stimulus to their self-maintenance is mechanical loading. Not only does physical activity help preserve bone mass, but it probably is an important factor in the maintenance of adequate remodeling, without which fatigue damage will accumulate. It is difficult to increase bone mass very much by increasing physical activity; unfortunately, it is easy to lose mass with inactivity. So maintaining load-bearing activity is critical.

Finally, a brief word about pharmacotherapy. Recent promising reports describing the use of bisphosphonates indicate that these agents may have a useful role in the management of patients who already have the disorder.[23,24] Many years of experience have been accumulated with their use in Paget's disease, and so their long-term safety is reasonably assured. They are, however, remodeling suppressors, and their long-term effects in persons who already have compromised skeletal strength will have to await further study.

Fluoride is another issue. Riggs is cautious, which is understandable, given his recently reported experience in which fluoride did not decrease fracture incidence despite a nearly 40% increase in bone mass.[11] The protocol for his study, however, dictated not by the investigators but by the sponsoring agency, called both for what is now recognized to be a toxic dose of fluoride and for administration in a form that is known to produce excess gastrointestinal irritation.[25] The high peak blood concentrations produced by such therapy may well produce a degree of osteoblast toxicity that lower, but more sustained, blood levels do not. Certainly they will produce areas of hypercrystallinity in newly deposited bone mineral that will result in bone of different mechanical properties from bone mineralized out of a medium with a lower fluoride concentration. Fluoride is now approved for the treatment of osteoporosis in at least eight European countries and should not yet be counted out of the running in the US.

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Nitric Oxide, Nitrovasodilators, and L-Arginine—An Unusual Relationship

CERTAINLY ONE OF THE MOST interesting and potentially far-reaching discoveries that has taken shape over the past few years concerns a novel mammalian pathway that leads to the formation of nitric oxide from the amino acid L-arginine.[1] This unusual biochemical pathway brought together seemingly unrelated fields of research. Nitric oxide formation has now been found in a number of cell types, but the first and, until now, the most thorough characterizations have been in macrophages, endothelial cells, and cells of the central ner-

vous system. The action of nitric oxide generated through this pathway is dependent on the cell type that produces it. Endothelium-derived nitric oxide leads to vascular smooth muscle relaxation, and, although the debate continues, most of the evidence supports that nitric oxide is endothelium-derived relaxing factor (EDRF) and that it acts by activating the enzyme guanylate cyclase in smooth muscle tissue. In the central nervous system, nitric oxide acts in a manner similar to that from endothelial cells in that it signals an adjacent cell and in this case another neuron. Macrophage-derived nitric oxide is distinct in many ways from the other two cell types, and evidence supports a role for this nitric oxide in the cytostatic activity that is a critical function of these activated immune system cells.

The history of this pathway is relatively short and illustrative of the convergence of apparently disparate research areas. During the late 1970s, a number of key observations concerning guanylate cyclase were made whose overall importance and relationship to this pathway became clear later. In particular, Murad and colleagues showed that nitric oxide activates guanylate cyclase,[2] Craven and DeRubertis showed that this nitric oxide activation is dependent on heme,[3] Ignarro and co-workers found that nitric oxide leads to the relaxation of smooth muscle,[4] and Furchgott and Zawadzki showed that the endothelium was required for vascular smooth muscle relaxation.[5] Also in the late 1970s, a number of laboratories involved with studies of the brain contributed to the growing idea that nitric oxide activated guanylate cyclase and that arginine and glutamate stimulated guanylate cyclase activity as well. In the early to middle 1980s, studies carried out with macrophages provided some key pieces to the puzzle. In experiments related to the endogenous formation of carcinogenic *N*-nitrosamines, Tannenbaum and coworkers showed that mammals were capable of nitrate synthesis.[6] Continuing studies in that laboratory showed that nitrate urinary levels were significantly elevated after treating rats with immunostimulants such as lipopolysaccharide.[7] Then in 1985 Stuehr and Marletta determined that macrophages treated with lipopolysaccharide synthesized nitrite and nitrate,[8] and subsequent studies showed that lymphokines such as interferon gamma could also stimulate this synthesis.[9] The stage was set in 1987 and 1988 for the convergence of the findings in the central nervous system (CNS), endothelium, and immune system. Iyengar and associates and Hibbs and colleagues showed that the precursor for nitrite and nitrate in macrophages was L-arginine,[10,11] and, in addition, Iyengar and co-workers showed that the nitrite and nitrate were derived from the guanido group of arginine.[10] Hibbs and colleagues also showed that arginine was required for macrophage-induced tumor cell cytostasis.[12] The chemical identity of EDRF had been the subject of much speculation and research, but it was Moncada and associates who provided the first definitive proof that endothelial cells were capable of nitric oxide synthesis.[13] The solution instability of nitric oxide suggested that it was likely to be the intermediate that led to nitrite and nitrate in the macrophage studies mentioned above. Marletta and co-workers and Hibbs and associates provided direct proof that this was indeed the case.[14,15] Moncada and colleagues then showed that the endothelial cell nitric oxide was derived from L-arginine,[16] and Garthwaite and co-workers showed that nitric oxide was produced by glutamate stimulation of *N*-methyl-D-aspartate receptors.[17] It was now clear that the L-arginine to the nitric oxide pathway was the common theme in all of these observations.

An immediate outcome of these findings is that they provide a rational mechanism for the action of nitrovasodilators such as nitroglycerin. Nitroglycerin is known to decompose to nitric oxide under biologic conditions, and although the series of reactions is mechanistically complex and not completely understood, the reaction is facilitated in the presence of thiols such as glutathione. Therefore, nitroglycerin and related nitrovasodilators act by circumventing the enzymic generation of nitric oxide. It is particularly satisfying that the study of this pathway has led to an understanding of the mechanism of action of such an important class of drugs in use for more than 100 years and, perhaps more important, points the way toward the design of agents that could potentially mimic the pathway more successfully. As mentioned, there is not uniform agreement on the identity of EDRF. The debate has focused on the properties of EDRF generated from stimulated endothelial cells in a smooth muscle bioassay versus the action of nitric oxide and various *S*-nitroso compounds in the same bioassay. The answer will come from studies on the enzymology of the pathway in the various cell types, but at this point it appears that it is nitric oxide that is released by the enzyme. The solution decomposition of nitric oxide generates a number of products that readily nitrosate thiols and amines which can, especially in the case of *S*-nitroso compounds, cause smooth muscle relaxation.

The regulation and biochemistry of the pathway are two aspects under investigation at present. The results of these studies are likely to provide key pieces of information with regard to the potential for cell-type-specific drug interactions. Some important differences have already emerged where it is clear that the macrophage is distinct from the other cell types in a number of respects. Consistent with the function as a signaling agent, nitric oxide synthesis occurs in a burst in both endothelial and CNS cells. The activity in these cells requires calcium ion (Ca^{2+}), and a report of the enzyme purification from rat brain showed a requirement for Ca^{2+}-complexed calmodulin and a single polypeptide of 150,000 daltons.[18] Because of the similarities in the CNS and endothelial cell activity, it may well turn out that the endothelial cell also shows a calmodulin requirement. In macrophages the activity cannot be detected in untreated cells, but after stimulation and subsequent protein synthesis, the cells continuously synthesize nitric oxide. The activity in macrophages is enhanced by divalent metals with magnesium ion being the most effective; these metals are not required, however. In addition, the cofactor tetrahydrobiopterin has also been identified as a requirement in macrophages.[19,20] The most common role for this cofactor is in aromatic amino acid hydroxylation such as with phenylalanine hydroxylase. Experiments suggest that this is the function for this cofactor in macrophages. On the other hand, although there does not appear to be a low-molecular-weight cofactor involved in the CNS or endothelium, a relatively tightly bound pterin has not been ruled out. A number of arginine-related compounds have been shown to inhibit the reaction, most notably N^G-methyl-L-arginine, which inhibits the activity in all cells examined to date. L-Canavanine, however, appears to be a specific inhibitor for the macrophage and neutrophil, and, while not potent, it at least shows that selective inhibition is possible.

The stage is now set for exciting developments in the

application of this research to the development of new drugs. It is clear that substantial differences exist between the basic enzymology of the macrophage compared with that of the CNS and endothelial cells and in the regulation of the pathways in those cells. The extent to which these differences can be exploited will determine just how fruitful this area will be for drug development. It is difficult to see any advantage in the inhibition of the activity in endothelial cells, but inhibition of the activity in the macrophage may be useful in the treatment of endotoxic shock, inflammatory bowel disease, and arthritis and in cytokine therapy. The extensive use of the nitrovasodilators in the treatment of angina and, as brought out in the article in this issue by Ignarro, Ross, and Tillisch,[21] their potential in other cardiovascular diseases suggest that attention should be given to the development of new vasodilators with more controlled chemistry.

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